Determination of Acidic Herbicides in Surface Water by Solid-Phase Extraction Followed by Capillary Zone Electrophoresis

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Abstract

A rapid solid-phase extraction–capillary zone electrophoresis (CZE) method for determining 2,4-dichlorophenoxyacetic acid, 4-(2,4-dichlorophenoxy) butyric acid, and 2,4,5-trichlorophenoxyacetic acid in real water samples is described. Factors affecting the recoveries and detection of the targets are investigated. With samples being acidified to pH 2 and salted by sodium sulfate to 2% (w/w), an average recovery of greater than 85% is obtained using ethyl acetate as the eluent on an octadecylsilane-bonded silica cartridge. A running buffer of 5mM sodium tetraborate in a water–acetonitrile mixture (70:30, v/v) adjusted to pH 9 is employed in the CZE analysis, and the targets can be analyzed within 7 min with good reproducibility and acceptable sensitivity. The method is suitable for detecting herbicide residues of sub-parts-per-billion levels in surface water. A local pond water is analyzed, and the concentrations of 2,4-dichlorophenoxyacetic acid and 4-(2,4-dichlorophenoxy) butyric acid are detected to be 0.27 ± 0.03 ppb and 0.61 ± 0.08 ppb, respectively.

Introduction

Chlorophenoxy acids are herbicides widely used for controlling weeds in agriculture. Because most of them are readily dissolved in water, they can easily enter into surface or ground waters through natural drainage or filtration (1). These generate toxicity to human beings and aquatic organisms. Therefore, it is important to determine the presence of these herbicides in drinking or surface waters.

The most commonly used method is EPA Method 515.1, which uses liquid–liquid extraction combined with gas chromatography (GC)–electron capture detection to detect acidic herbicides in drinking water (2). However, derivatization of the acidic herbicides is needed because some of them are not volatile or stable under the operating temperature (8). Recently, solid-phase extraction (SPE) combined with high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) has been studied to determine them (3–10). Compared with GC, no derivatization of the herbicides is needed in HPLC or CE. When using HPLC to analyze real samples, several different SPE columns need to be used together to clean up the sample solutions because there exists a lot of interfering compounds (such as humic and fulvic acids) in surface water. Capillary zone electrophoresis (CZE) is a high-efficiency separation technique based on a different separation mechanism from HPLC. Because the separation is based on mass–charge ratios, the neutral and positively charged compounds in surface water will not interfere with the determination of the negatively charged herbicides. This advantage of CZE makes the pretreatment of sample solutions by a single SPE column possible. Several workers have studied the use of CZE to separate acidic herbicides in standard mixtures (11,12) and spiked lake water samples (15), but so far no report has been found using SPE–CZE for the determination of acidic herbicides in real surface water samples.

The aim of this work is to investigate factors affecting the recoveries of analytes during the SPE procedure and separation of the analytes by CZE and to develop a method for detecting acidic herbicides in surface water.

Experimental

Chemical

2,4-dichlorophenoxyacetic acid (2,4-D); 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB); and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were products from AccuStandards (New Haven, CT). Sodium sulfate anhydrous and sodium tetraborate anhydrous were purchased from Fluka (Buchs, Switzerland). HPLC-grade acetonitrile and ethyl acetate were products of J.T. Baker (Phillipsburg, NJ). HPLC-grade methanol was obtained from Fisher Scientific (Fair Lawn, NJ). The deionized water in the experiments was prepared by a Milli-Q system (Millipore, Bedford,

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MA). All of the other chemicals were ordered as highest purity.

Instrumentation

Polyimide-coated fused-silica capillaries with an inner diameter of 50 µm and an outer diameter of 360 µm purchased from Polymicro (Phoenix, AZ) were used. Solutions were filtered with 0.22-µm Millipore filters. SPE was performed on a 500-mg Varian (Harbor City, CA) octadecylsilane (C_{18})-bonded silica cartridge. A CE-L1 controlling unit (CE Resources, Singapore, Republic of Singapore) equipped with a Linear Instrument (Reno, NV) UVIS 200 detector was employed to perform CZE experiments. The instruments were interfaced to a computer, and CSW17 software (DataApex, Prague, Czech Republic) was used for data acquisition and evaluation. Samples were introduced to the capillary from its anodic side by a pressure of 0.3 psi for 10 s.

CE procedure

The new fused-silica capillary was first treated by rinsing with methanol for 30 min, followed by deionized water for 1 h and then by 0.1M sodium hydroxide for at least 5 h. Every day before experiments, the capillary was flushed with 0.1M NaOH for 20 min, then with deionized water for 5 min, and finally with buffer for 20 min. It was flushed with running buffer for 1 min between two consecutive runs or when any poor performance (such as poor peak shape or noisy baseline) was observed. All of the previously mentioned solvents were filtered with a 0.22-µm filter before use.

SPE procedure

The following procedures were employed in the experiment unless otherwise stated.

Stock solutions of the herbicides of 20 ppm each were prepared each day. Milli-Q water and surface water were spiked to desired concentrations with the stock solutions. All solutions were added to sodium sulfate (2%, w/w) and acidified to pH 2 by 4M hydrochloric acid.

The cartridge was first cleaned by passing it through 5 mL methanol and consequently drying it with ultrapure nitrogen for 5 min. Then, it was rinsed with another 3 mL methanol followed by 10 mL deionized water. After being preconditioned, the sorbent was not allowed to dry until the sample loading procedure finished.

The herbicide-containing solution was passed through the SPE cartridge at a flow rate of 10 to 20 mL/min under positive pressure. After the cartridge was dried with nitrogen for approximately 2 min, the adsorbates were eluted using 2 mL of an organic solvent (ethyl acetate, methanol, or acetonitrile as stated). The effluent was then heated to 50°C and evaporated to dryness using a gentle stream of nitrogen. In order to avoid unexpected particles blown into the solution, the nitrogen delivery cable was connected to a filter (0.22 µm). The residue was dissolved in a 0.1-mL mixture of water and methanol (50:50, v/v) before the CZE analysis.

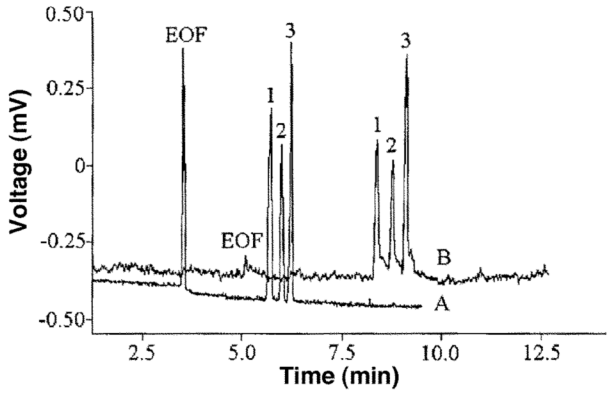

Figure 1. Comparison of different buffers. The sample was Milli-Q water spiked with the standards to 10 ppm. The capillary had a 50-µm i.d. and 41.5 cm/32 cm total/effective length. The applied voltage was +12 kV, and the UV detection was at 230 nm. The buffers were: (A) 5mM sodium tetraborate in a water–acetonitrile mixture (70:30, v/v) adjusted to pH 9 by 4M hydrochloric acid and (B) 5mM sodium tetraborate in a water–methanol mixture (70:30, v/v) adjusted to pH 9 by 4M hydrochloric acid. The peaks were: (1) 2,4-DB; (2) 2,4,5-T; and (3) 2,4-D.

Results and Discussion

Optimization of CZE conditions

Concentration of organic components

For CZE, it is important that the analytes are soluble in the buffer. Because the solubility of 2,4-DB in water is very low, organic solvents should be added into the aqueous buffer. Introduction of organic solvents also affects CZE parameters. One effect is that the conductivity of the buffer decreases, and therefore a higher separation voltage can be applied without significantly increasing Joule heat during the analysis (thus favoring high separation efficiency and rapid analysis). However, the electroosmotic flow (EOF) decreases with the addition of organics. Because under this operation mode the analytes migrate in the direction opposite to the EOF and they are "drawn out" by EOF, the analysis time is very sensitive to the change of EOF. From our observation, the overall effect of organic addition is to increase the analysis time. The objective of this work was to find a balance between the solubility of the target analytes and the analysis time. Both methanol and acetonitrile were tested as organic additives, but buffer-containing acetonitrile offered better performance (Figure 1). From an acetonitrile concentration of 10% (v/v) and above, both peak shapes and the reproducibility of peak areas (A_p) were good for the analytes. When the concentration was beyond 50%, there was a significant increase of analysis time (from approximately 7 min at 20% to approximately 17 min at 60%). In this study, the concentration of acetonitrile was chosen to be 30% (v/v).

Buffer pH and concentration of sodium tetraborate

The alkali buffer not only favors the deprotonation of the targets but increases EOF and thus decreases the analysis time. A sodium tetraborate solution was chosen as the running buffer, and it was adjusted by 4M hydrochloric acid to pH 9. From the sodium tetraborate concentration of 2mM onward, well-shaped and baseline-separated peaks corresponding with the analytes were observed even for herbicides of sub-parts-per-million levels, which suggested that adsorption of the analytes on the capillary

surface was not a serious problem under such conditions. The concentration of the buffer was chosen as 5mM because the EOF decreased as the buffer concentration increased, causing the analysis time to increase from approximately 7 min at 5mM to approximately 13 min at 15mM. There was no need for the higher buffer concentration in this experiment because the concentration of the analytes introduced into the capillary was only 2 ppm for the standards and lower for the real samples (either of them would be less than 1/100 of the buffer concentration). This was confirmed by the lack of a more significant stacking of the analytes at a higher buffer concentration from the assessment of both the peak heights and areas.

Optimization of SPE conditions

Eluent and its influence on analysis

Methanol, acetonitrile, and ethyl acetate are commonly used eluents for SPE, and they were tested for their feasibilities as eluents in this experiment. The recoveries of herbicides were above 90% (n = 5) for all of the solvents when each eluent was spiked with the herbicides to 0.2 ppm each. However, precipitation was observed when an aqueous solution containing the targets of 20 ppm each was mixed with the pure acetonitrile to 10:90 (v/v of aqueous to organic). It disappeared when water was added to the ratio of 40:60. Methanol showed slightly higher solubility for the acidic herbicides than ethyl acetate (on average approximately 2% recovery), but it was also a good solvent to humic acid as observed. Humic and fulvic acids are the main interfering matrices for the determination of trace herbicides in real water samples. It has been pointed out by other workers (14) and also observed in our experiment that ethyl acetate, when used as an eluent, was effective in reducing the concentration of humic acids in the effluent. Although it was reported that the addition of methylene dichloride into ethyl acetate could enhance the recoveries of the polar extractants, there was, to our observation, little improvement of recoveries of the analytes in ethyl acetate after 10–30% (v/v) methylene dichloride was added. Pure ethyl acetate was used as an eluent in our experiment because it could offer satisfactory recoveries without the addition of methylene dichloride, which might be potentially more harmful to the human body.

Because CZE employs a different separation mechanism from HPLC, GC, or micellar electrokinetic chromatography, the concentration of humic acid may affect the detection of the phenoxy acids to different extents. We found that 5 ppb of acidic herbicides spiked in real samples could be detected by CZE without the elimination of humic acid during the extraction procedure (Figure 2). For real samples containing sub-parts-per-billion levels of targets, ethyl acetate should be used because of the highly concentrated matrix.

Highest recoveries of all the analytes could be obtained with an elution volume larger than 1.5 mL, thus 2 mL eluent was used in the experiments.

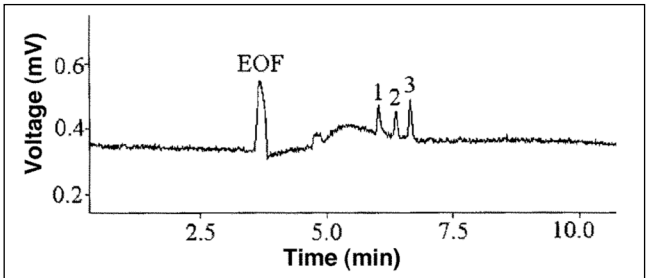

Figure 2. Electropherogram of herbicides spiked in surface water. The sample was 200 mL surface water spiked with herbicides to 2.5 ppb each, extracted with C18, and then eluted by methanol. The dried residue was dissolved in a 0.5-mL water–methanol (50:50, v/v) mixture. The buffer was 5mM sodium tetraborate in a water–acetonitrile mixture (70:30, v/v) adjusted to pH 9 by 4M hydrochloric acid. Other conditions were the same as in Figure 1.

Salt-out effect and concentration of sodium sulfate

The recoveries of the herbicides were not satisfactory, even after the pH of the sample was adjusted to 2 with 27.1% for 2,4-D; 55.3% for 2,4,5-T; and 71.7% for 2,4-DB. Some inorganic salts such as potassium chloride or sodium chloride (13) were added to the sample solution to improve the retention of the polar analytes onto the solid phase in order to increase the recoveries of the herbicide. In this work, sodium sulfate was added to the sample and the influence of concentration on the recoveries of the three targets was studied. A sodium sulfate concentration of higher than 0.5% (w/w) could offer maximum recoveries (higher than 95%) for all the analytes. In view of the complexity of the real samples, solutions were added by sodium sulfate to 2% (w/w) before passing through the cartridge.

Influence of pH

The pK_a values of 2,4-D; 2,4-DB; and 2,4,5-T are 2.87, 4.95, and 2.83, respectively. An acidic environment will theoretically favor their adsorption on the C_{18} sorbent. Although not as significant as the addition of salt, the pH value did have some effect on the adsorption of the acids. The recoveries of 2,4-D; 2,4-DB; and 2,4,5-T increased with decreasing pH (from 85.2%, 81.5%, and 79.1% at pH 7 to 97.2%, 99.2%, and 100.1% at pH 2, respectively). However, we did not find the obvious elimination of the humic and fulvic acids under neutral conditions as observed by other authors (7). The sample solution was acidified to pH 2 before extraction because further acidification may cause hydrolysis of the Si-O-C bond of the sorbent.

Table I. Validation of the SPE–CZE Method

	%Recovery ± %RSD (n = 5)*			%RSD of t_m	%RSD of A_p	
	0.5 ppb	5 ppb	10 ppb	(n = 15)	(n = 5)†	LOD (ppb)
2,4-DB	96.7 ± 7.0	100.7 ± 5.3	98.0 ± 4.6	0.29	1.4	0.12
2,4,5-T	97.0 ± 8.4	96.4 ± 5.4	99.9 ± 6.1	0.17	1.9	0.15
2,4-D	103.5 ± 6.2‡	98.4 ± 4.6	98.1 ± 3.6	0.26	1.9	0.09

* Evaluated based on 400 mL deionized water spiked to the concentrations stated.
† For the 5-ppb extracts.
‡ The spiking concentration was 0.2 ppb.

Validation of the method

Standard solutions of concentrations corresponding with 2, 5, 10, 15, and 20 times the limits of detection (LOD) (signal-to-noise = 3) in 400 mL

water before the SPE procedure were used for the study of linearity. The calibration curve of A_p (y) (mV·S) versus concentration (x) (ppb) for each herbicide was constructed, and their regression equations and correlation coefficients (r) were calculated as $y = 0.3147x - 0.0193$ ($r = 0.9994$) for 2,4-D; $y = 0.2715x - 0.0148$ ($r = 0.9991$) for 2,4-DB; and $y = 0.1766x + 0.0066$ ($r = 0.9949$) for 2,4,5-T. In order to assess the reproducibility of the calibration curve, a three-day validation was carried out. In each day, all five of the freshly prepared standard solutions were measured three times. Each herbicide was evaluated with all nine of the curves. The correlation coefficients for the linear best fit were no less than 0.992, and the relative standard deviation (RSD) for the slope and the intercepts were no more than 4.21% and 5.17%, respectively.

Herbicide solutions of 400 mL each of different concentrations were employed to evaluate the recoveries in the SPE procedure, the RSD of the migration time (t_m), and A_p in CZE. Table I shows that the SPE–CZE method is of good repeatability and high sensitivity, and it can be used in analyzing herbicides of sub-parts-per-billion levels. The method may be used in detecting herbicides of a lower concentration because the sample volume can be as high as 1000 mL without a significant decrease in the percent recoveries. The method was also assessed for the feasibility of detecting herbicides in a real water sample. Compared with the unspiked real water sample as the control, recoveries between 86.1% and 107.0% were obtained from 400-mL samples spiked with a 0.2- to 2.0-ppb herbicide each.

Real sample analysis

The method developed was applied to the determination of the concentrations of acidic herbicides in local pond surface water (Normanton Park, Singapore). Although the baseline after EOF was not very stable because of the high concentration of the interfering matrix, the species present could still be quantitatively identified by t_m (also by spiking in our experiment) and determined by A_p (Figure 3). Two herbicides (2,4-DB and 2,4-D) were identified in the water sample and their concentrations were 0.61 ± 0.08 ppb ($n = 3$) and 0.27 ± 0.03 ppb ($n = 3$), respectively. For comparison, methanol was used as an eluent in the SPE procedure (Figure 3). The high noise level of the baseline suggested that methanol is poorer in selectivity than ethyl acetate. The extract eluted by ethyl acetate was also analyzed by a Waters HPLC system (a Waters 600E controlling unit and a Waters 486 UV detector were interfaced to a computer; a Spherisorb ODS1 column (150 × 4.6 mm) was used; the eluent was 6mM nitric acid in a 60:40 (v/v) methanol–water mixture; and the flow rate was 0.6 mL/min). The conditions were similar to those in a previous publication (16). Before analysis, the system was calibrated with standard solutions, and the linearity for each herbicide was determined from the A_p values of different concentrations over the range of 0.4 to 6 ppm (equal to 0.1–1.5 ppb in 400 mL water before the SPE procedure). The relative coefficient values were all better than 0.99. The concentrations of 2,4-DB and 2,4-D were found to be 0.74 ± 0.11 ppb ($n = 3$) and 0.21 ± 0.05 ppb ($n = 3$), respectively. It was observed that the baseline of the HPLC chromatogram was worse than that in the CZE electropherogram. The poor baseline might be attributed to the interference from humic and fulvic acids. Both HPLC and CZE methods do not require derivatization of the acidic herbicides. However, compared with SPE–HPLC, the SPE–CZE method presented in this study may be a better alternative or complement to Method 515.1 because interferences can be more easily alleviated.

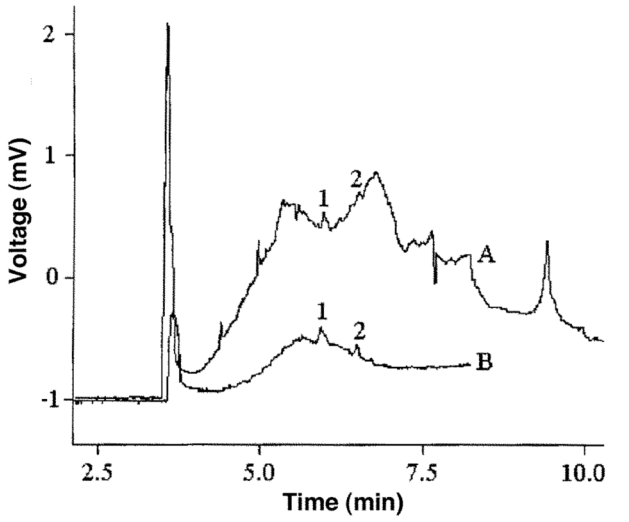

Figure 3. Analysis of a real sample. The sample was 400 mL local surface water. The dried residue was dissolved in 0.1 mL water–methanol (50:50, v/v). The eluents were: (A) methanol and (B) ethyl acetate. The peaks were (1) 2,4-DB and (2) 2,4-D. Other conditions were the same as in Figure 2.

Conclusion

CZE is an effective method in analyzing charged species. Besides fast analysis and high efficiency, it may alleviate the problems of interferences encountered by HPLC or GC. SPE–CZE is potentially a useful approach in determining acidic herbicides in the environment. We think some advances (such as a well-matched SPE eluent and CZE buffer and improvement in detection sensitivity) will help to extend its application in routine analysis.

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